Thiophene analogues of phenotiazine neuroleptics. Physicochemical and biological properties of thienobenzothiazines

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CHAPTER X
SUMMARY AND CONCLUSIONS

From the investigations described in this thesis the thienobenzothiazines were found to be a useful tool to study the influence of certain properties on neuroleptic activity of compounds which are comparable in parameters affecting the accessibility to a site of action. In this chapter we shall briefly review our findings and make an attempt to draw some conclusions from them. In this respect it must be realized that this report is an account of ongoing studies, obviously not permitting definite conclusions. The results of the study of sixteen thiophene analogues of phenothiazines and the six parent compounds have been summarized in Table X-1.

The synthesis of these thienobenzothiazines was described in Chapter II, in which some spectroscopic and physical properties are summarized in Table II-2.

Aqueous dissociation constants in physiological salt were determined with the potentiometric titration method of Levy and Rowland (Chapter III). Due to problems encountered with centrifugation of the titration mixture, pKa values of the dimethylaminopropyl compounds are somewhat less accurate than those of the hydroxyethylpiperazine derivatives.

Determination of apparent partition coefficients in octanol/water at pH 7.4 (μ = 0.15) by a double extraction procedure gave very good results (Chapter IV). It was shown that at this pH partitioning is essentially due to the base. Therefore, true partition coefficients of the unionized species could be calculated with the aid of pKa values.

The lowering of the surface tension of phosphate buffer pH 7.4 of the same ionic strength was measured with the ring detachment method with concentrations of the compound at which the ionized form was devoid of activity (Chapter V). For the phenothiazines it was demonstrated that a ranking of relative surface activities of the base and of the ion was approximately the same, the latter being a few hundred times less active. The concentration of base causing a surface pressure of 5 dyne/cm was again calculated with the aid of pKa values. We expressed both the parameters, representing partitioning and surface activity, in terms of the unionized species, because the base will be transported and distributed through lipophilic and hydrophilic phases.

Half wave oxidation potentials were determined in 6 N H_2SO_4 by polarography,

as a measure of radical cation formation (Chapter VI).

A comparison of these physicochemical properties (Table X-1), reveals some interesting features. In all cases studied the [3,4-b]-isomers 2, 4, 7, 10, 12 and 15 stand out because of their resemblance with the corresponding phenothiazines. This similarity also applies to the spectroscopic data as was discussed in Chapter II. The other two thienobenzothiazine isomers are very like each other in all respects, except for the irreversible oxidation waves of the [3,2-b]-isomers 5, 8, 13 and 16. Hence the resemblance between the [3,4-b]thienobenzothiazines and phenothiazines on the one hand, and the [2,3-b]- and [3,2-b]thienobenzothiazines on the other hand, is more pronounced than between the members of each set of thienobenzothiazine isomers. Differences in biological activity were established by measurement of the degree of antagonism to amphetamine stereotypy in rats (Chapter VII), of the potencies to increase HVA-levels in rat striatum (Chapter VIII) and, as yet only for the thiophene analogues of fluphenazine, of the inhibitory effects on dopamine-stimulated adenylate cyclase activity in rat striatal homogenates (Chapter IX). Of course it is still doubtful whether these parameters really reflect neuroleptic activity. The rise in HVA-level in rat striatum correlates very well with the degree of amphetamine antagonism, but it is not known if these parameters are a measure of antipsychotic potency or rather of the ability of the compound to produce side effects, in particular Parkinsonian-like symptoms. Clozapine and, to a lesser extent thioridazine, are examples of neuroleptics, which are claimed to combine low potency in amphetamine antagonism with weak extrapyramidal activity. However, the good correlation we found between HVA-increasing capacities and clinical doses of fourteen neuroleptics, including clozapine and thioridazine, emphasizes the value of measurement of HVA increase in rat striatum for predicting neuroleptic efficacy. Moreover, for compounds belonging to one class of neuroleptics, like the thienobenzothiazines and phenothiazines, a certain biological response is likely to represent a similar type of activity. Consequently it seems plausible to assume that the parameters we determined reflect relative neuroleptic potencies.

All thienobenzothiazines are less active than the corresponding phenothiazines. Striking differences were found within each set of thienobenzothiazine isomers: [3,4-b]- and [2,3-b]-isomers are about equally active, while the [3,2-b]-isomers exhibit much lower potency. Regarding the physicochemical similarity between the phenothiazines and [3,4-b]thienobenzothiazines the decrease in activity of the latter compounds is surprising. All

Table X-1

Survey of physicochemical and biological parameters of phenothiazines and thienobenzothiazines

Compound[1]	dissociation[2] constant	partition[3] coefficient	surface[4] tension	half wave[5] potential	amphetamine-[6] antagonism	HVA-[7] increase	inhibition of[8] adenylate cyclase activity
Promazine	9.6	4.8	2.5	391	120	217	
1	9.1	4.5	9.4	190	-	480	
2	9.6	4.8	3.1	390	60	480	
Chlorpromazine	9.5	5.5	0.9	400	0.9	5.8	
3	9.0	5.0	2.7	240	30	36.0	
4	9.4	5.5	0.8	450	30	19.2	
5	9.2	4.9	2.0	270	-	350	
Trifluprom azine	9.5	5.8	0.2	460	0.9	1.4	
6	8.5	5.1	2.1	280	1.8	6.1	
7	9.2	5.5	0.3	520	7.5	9.2	
8	8.9	5.3	0.8	310	-	30.1	

Table X-1

(continued)

Table X-1

(continued)

Compound[1]	dissociation constant[2]	partition coefficient[3]	surface tension[4]	half wave potential[5]	amphetamine-antagonism[6]	HVA-increase[7]	inhibition of adenylate cyclase activity[8]
"Phenazine"	7.9	3.5	35.6	-	-	-	
9	7.7	3.2	65.9	180	-	18.9	
10	7.7	3.4	53.3	390	1.3	13.7	
Perphenazine	7.9	4.3	7.4	410	0.16	0.25	
11	7.8	4.2	13.5	230	1.28	3.3	
12	8.0	4.4	6.3	460	0.64	1.5	
13	7.8	4.1	10.5	260	-	20.5	
Fluphenazine	7.9	4.6	1.9	470	0.16	0.16	0.016
14	7.8	4.4	3.6	300	0.32	0.75	0.009
15	7.9	4.6	1.9	520	0.32	0.54	0.010
16	7.7	4.4	3.6	320	-	4.0	0.21

1) coding of the compounds refers to the last page.
2) pKa^c in water (μ=0.15, 25°C) (Chapter III).
3) log P octanol/water (μ=0.15, 25°C) (Chapter IV).
4) concentration of base (μmol) causing a surface pressure of 5 dyne/cm at phosphate buffer pH 7.3 (μ=0.15, 25°C) (Chapter V).
5) $E_{1/2}$ in mV vs. SCE in 6N H_2SO_4; phenothiazines in 12N H_2SO_4 (Chapter VI).
6) lowest active dose (μmol/kg) which antagonizes amphetamine stereotypy (Chapter VII).
7) dose (μmol/kg) causing a striatal HVA concentration, three times control level ED_{300} (Chapter VIII).
8) inhibition constant K_i (μmol) for dopamine-sensitive adenylate cyclase (Chapter IX).

parameters studied being virtually the same, we might conclude that this difference is caused by differences in conformation or by differences in metabolism. Although we have as yet no stereochemical data of the thienobenzothiazines, we do not expect great structural differences between these compounds and the phenothiazines. However, folding of the tricyclic ring system and the conformation of the side chain might be affected by the introduction of a thiophene ring. The preliminary results of the study on the inhibitory actions of the [3,4-b]thienobenzothiazine isomer 15 and fluphenazine on dopamine-stimulated adenylate cyclase activity may be considered as a support for the suggestion of metabolic differences, assuming that this enzyme system is an in vitro model of the dopamine receptor. In vivo, fluphenazine is more active than the [3,4-b]thienobenzothiazine 15 but the in vitro activity is approximately the same. Obviously this phenomenon cannot be explained by physicochemical differences, to which the discrepancy between relative in vivo and in vitro activities of phenothiazines and butyrophenones is sometimes attributed. The lower in vivo activity of the [3,4-b]thienobenzothiazine might be due to differences in metabolism, possibly causing a lower concentration at the site of action, or to a diminished potency of possible active metabolites. As the half wave potentials of both compounds, reflecting radical cation formation, are virtually the same, differences in oxidative metabolism, if any, are in subsequent steps of the oxidation process. It is conceivable that these proceed by a different mechanism in case of the thienobenzothiazines.
As already mentioned, the [2,3-b]- and [3,2-b]-isomers are comparable with respect to all physicochemical properties studied, except for their electrochemical behavior. The [3,2-b]-isomers, showing the same half wave potentials as the [2,3-b]-isomers, were all irreversibly oxidized in 6 N H_2SO_4, which might also have consequences for their metabolic fate. However, in this case the lower potency of the [3,2-b]-isomers in vivo, was also found in vitro. Further study on their sterochemistry and metabolism is required to be able to explain this interesting difference between [2,3-b]- and [3,2-b]-isomers.
We can make some additional remarks on the relevance of some physicochemical parameters. Occasionally a relationship was suggested between the neuroleptic activity of a compound and a certain physicochemical property like partition coefficient, surface activity or radical cation formation. The results of our study indicate that such a simple correlation is not very likely, or

at least difficult to make, because of simultaneous differences in other properties.

One of the major problems in studying structure-activity relationships of central nervous system agents is the lack of knowledge about the events between the application of the drug and the measurement of an effect. Incorrect conclusions can be drawn from observations with compounds, which may reach a site of action in quite different concentrations. One can circumvent this problem by measurement of in vitro activities or local application of the drug. We have tried to overcome this difficulty by studying closely related compounds which can be expected to have comparable physicochemical properties. Our results show that the [3,4-b]thienobenzothiazines with corresponding phenothiazines, and the isomeric [2,3-b]- and [3,2-b] thienobenzothiazines are two sets of such drugs. Further study of these compounds might contribute to the elucidation of the mode of action of phenothiazine neuroleptics.